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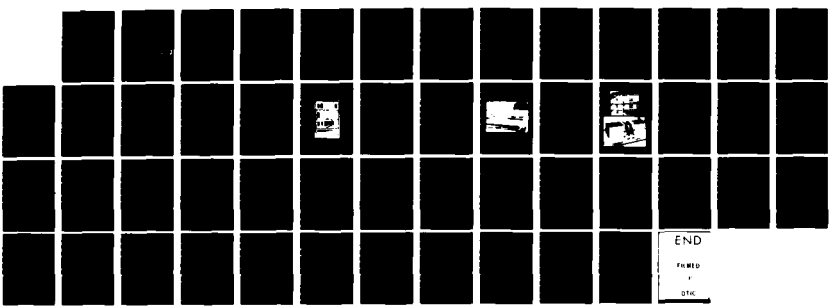

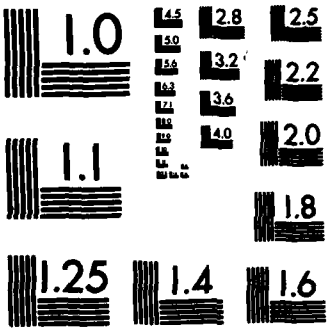

JOINT FAO/IAEA DIVISION OF ISOTOPE AND RADIATION
APPLICATIONS OF ATOMIC ENERGY
FOR FOOD AND AGRICULTURAL DEVELOPMENT

INTERNATIONAL ATOMIC ENERGY AGENCY -
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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Application of the Sterility Principle for Tsetse Fly Eradication or Control

(Revised from 1972 Atomic Energy Review, Vol. 10, No. 1)

14 August 1981

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Joint FAO/IAEA Division of Isotope and Radiation
Applications of Atomic Energy
For Food and Agricultural Development

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Vienna, Austria

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Table of Contents

I. POTENTIAL FOR APPLICATION OF THE STERILITY PRINCIPLE IN A TSETSE CONTROL OR ERADICATION PROGRAMME
- A. Principles of the Method
- B. Theoretical Models and Field Trials of the Method
- C. Advantages of the SIT
- D. Disadvantages of the SIT
- E. Additional Research Requirements

II. CHARACTERISTICS OF THE RELEASE SITE
- A. Size of the Release Area
- B. Isolation of the Release Site
- C. Prior Reduction of Tsetse Populations
- D. Recommendations

III. ECOLOGY AND DISTRIBUTION
- A. Local and Seasonal Variations in Distribution
- B. Ecological Problems

IV. METHODS OF MASS REARING
- A. Environmental Conditions
- B. Adult Cages
- C. Maintenance and Handling Methods
- D. Feeding Methods
  1. Feeding on Live Animals (in vivo)
  2. Artificial Methods of Feeding (in vitro)
- E. Animal Housing and Management
- F. Mass Rearing Facilities
  1. In vivo feeding
  2. In vitro feeding
  3. Location
  4. Initiation of Colonies and Precautions
- G. Recording Results
- H. Mathematical Models

V. STERILIZATION PROCEDURES
- A. Sterilization of Adults
- B. Sterilization of Pupae
- C. Effects on Survival and Competitiveness
- D. Chemosterilization vs. Radiation
- E. Optimal Level of Sterility
- F. Other Genetic Approaches
- G. Recommendations

Table of Contents (cont.)

VI. DISTRIBUTION METHODS FOR STERILE MALES
- A. Adult Shipment
- B. Pupal Shipment
- C. Storage
- D. Releases
- E. Recommendations

VII. FIELD ASSESSMENT TECHNIQUES
- A. Recommendations

VIII. GENERAL RECOMMENDATIONS

IX. REFERENCES AND SELECTED BIBLIOGRAPHY

X. LIST OF WORKING PAPERS PRESENTED AT ANTWERP

APPLICATION OF THE STERILITY PRINCIPLE FOR TSETSE FLY ERADICATION OR CONTROL[1/]

JOINT FAO/IAEA DIVISION OF ISOTOPE AND RADIATION APPLICATIONS OF ATOMIC ENERGY FOR FOOD AND AGRICULTURAL DEVELOPMENT

ABSTRACT. This review deals with the conditions required for the successful application of the sterile insect technique (SIT). The prerequisites necessary for conducting field trials are outlined, information currently available is summarized and the types of information needed are itemized. Primary consideration focusses on the applicability and feasibility of SIT as an integral part of tsetse fly suppression.

I. POTENTIAL FOR APPLICATION OF THE STERILITY PRINCIPLE IN A TSETSE CONTROL OR ERADICATION PROGRAMME

A. Principles of the method

When sufficient numbers of sterile male insects are released into a wild population so that they can disperse and compete with the fertile males of the natural population, they can reduce the natural rate of population increase to less than one-fold per generation, thus reducing the population to zero within a predictable length of time. The method is most efficient in controlling insect pests whose populations are normally at a low level, on the decline, or have been markedly reduced by other causes. In instances where insects are extremely abundant, it may be necessary to reduce their numbers initially by the use of appropriate chemical, biological or other techniques.

The application of the Sterile Insect Technique (SIT) for eradication from an area presupposes a knowledge of the insect's ecology, population dynamics and ethological and ecological characteristics. Among the major requirements are: insectaries capable of producing at reasonable cost the required numbers of males to sterilize and release, efficient handling and sterilizing techniques that do not adversely affect the competitiveness of the treated individuals, and a suitable method for releasing the males into the environment.

The adaptation of the sterile insect technique to tsetse flies is confronted with unique problems because of the peculiar characteristics of these insects, such as: very low reproductive potential, longevity in the field and the vector potential of the released males.

1/ Revised from 1972 Atomic Energy Review, Vol. 10, No. 1, following recommendations of the research contractors attending the Joint FAO/IAEA Division Research Coordination Meeting on the Use of the Sterile Insect Technique for Tsetse Fly Eradication or Control, held in Antwerp, Belgium, 18-22 September 1978 and in Vom (BICOT), Nigeria, 11-14 November 1980.. The contributions of the Rijksuniversitair Centrum, Antwerp, and the Federal Government of Nigeria, respectively, to the success of these meetings are gratefully acknowledged.

B. Theoretical models and field trials of the method

Theoretical calculations by Knipling (1964) show in some detail what happens to a low-level tsetse fly population assumed to be about 200 adults per square mile, equivalent to 78 adults/km^2 (Table 1).

Table 1 Theoretical trend of a low-density population of tsetse flies subjected to sterile male releases at the rates and periods indicated. Average 100 males and 100 females per square mile (after Knipling, 1964).

Period (months)	Natural density at beginning of each period (males plus females)	Number of sterile males released each period	Estimated average ratio of sterile to fertile males	Assumed natural density at the end of each period
0-3	200	900 (300 per month)	3:1	75
4-6	75	450 (150 per month)	4:1	22
7-9	22	225 (75 per month)	7:1	4
10-12	4	112 (37 per month)	16.5:1	1

In calculating the theoretical effect of the sterile male releases, it is assumed that the released males are fully competitive with native males and exert full effect in reducing the reproductive potential of the total population. The assumption is also made that in the absence of control the natural population would increase by 50% from a low level each three months (each generation). Thus, in the absence of sterile male releases, the population trend would be as follows: first period, 200 flies per square mile; second period, 300 flies per square mile; third period, 450 miles per square mile; fourth period, 675 flies per square mile.

For this model, certain assumptions were made: the population is completely isolated, the maximum period of survival for adults is 90 days, a natural mortality of 50% is assumed in the natural population for both the first and second months of adult life, and the pupal period is assumed to be about 30 days with a pre-larviposition period of 15 days. From this model, we can see that an isolated low-level population of tsetse could theoretically be suppressed in about one year by successive releases of sterile males.

The theory was first tested under natural conditions in Africa with chemosterilized G. morsitans Westw. (Dame and Schmidt, 1970). Sterile males were released on an isolated island that had an original population of 300 to 600 flies of each sex. After 20 months of releases, native females were no longer present. However, a second species of fly, G. pallidipes Austen, remained on the island after the disappearance of G. morsitans. Although over 5 times as many sterile flies as postulated in Table 1 were released, this does not necessarily mean the model was wrong, for in the accompanying research it was found that untreated adults eclosing in the laboratory had a survival rate approximately 20% of normal when released in the field. The findings suggest that, under these conditions, it would require the release of 5 males to equal the normal effect of 1; the data fit the observations of the field trial remarkably well. In a separate field trial, the release of

chemosterilized G. morsitans that had been treated in the pupal stage was integrated with two aerial applications of insecticide. This treatment resulted in 96% control after 6 months and 99% control after 15 months.

Further confirmation of the SIT practicability was derived in a study in Tanga, Tanzania, involving an integrated approach of sterile males and insecticide (endosulfan). In this study, a 195-km^2 plot was sprayed twice at 28-day intervals to reduce initially the native populations of G.m. morsitans and G. pallidipes. Sterile G.m. morsitans males were released regularly between sprays and at a rate of 135/km^2 per month thereafter. After 7 months of releases, the G.m. morsitans density was only 6% of its initial pre-spray level, whereas that of the internal check G. pallidipes had returned to normal levels. The rate of 1.12 sterile males to each indigenous male was sufficient to keep G.m. morsitans populations in check for the 14 months of the study; this caused an overall reduction of 85-90% over pre-treatment levels despite unfavourable climatic conditions which reduced the efficiency of the barrier zone between the treated and untreated areas. Infiltration of flies from outside the test plot prevented the elimination of the test species, which was otherwise being completely controlled by the sterile males (Dame et al, 1980).

Studies carried out concurrently against G.p. gambiensis in Upper Volta were also highly successful (Cuisance et al, 1980). A non-residual insecticide (synthetic pyrethroid decamethrin-OMS 1988) was sprayed 11 and 14 days prior to the start of releases to reduce natural densities. A ratio of 3:1 sterile:wild was chosen for one site and 7:1 for another. G. tachinoides found cohabiting with G.p. gambiensis were used as an internal check. Within 6 months of releases, the G. tachinoides population had returned to its initial level, whereas the decreases at the 2 sites of G.p. gambiensis populations were 95% and 85% respectively. This species was subsequently eradicated along 11 km of the Guenaco and Guimpy Rivers (Cuisance et al, 1980).

C. Advantages of the SIT

In areas where the SIT could be utilized alone, this technique avoids contamination of the environment and selectively attacks one Glossina species, resulting in a minimum disturbance to the ecosystem. If an integrated use of insecticide is indispensable, this advantage may be lessened, but the contamination of the environment is, nevertheless, considerably lower (or insignificant) than what would have been encountered in a programme based entirely on chemical control. The volume of insecticide, timing of application and number of sprays necessary to lower the indigenous population can be reduced by 60% or more when used in conjunction with the SIT. When insecticides alone are employed against tsetse, the interval of sprays must be frequent enough to prevent larviposition and be continued until the soil-borne population has completed eclosion. Depending upon the residual toxicity of the pesticide used, the number of sprays could vary from 2 to 8 consecutive applications.

In contrast to treatment with insecticides, which are often most efficient against high insect populations, the SIT is much more efficient

against populations which are very low. The more environmentally safe use of lower volume, short residual insecticide can provide adequate knockdown of tsetse flies to allow the SIT to eleminate the remaining population. Because of this, the two methods are ideally suited to be integrated for less costly control or eradication of tsetse fly populations.

Unlike the majority of other Diptera, Glossina species have an extremely low reproductive potential and a low rate of increase per generation. Therefore, fewer sterile males would be required.

The density of the vegetation in the environment, which can be a serious obstacle to the application of insecticides, presents no problems in the dispersal of the sterile insects released.

When population density is low, the chances of eradicating the species are theoretically better with the SIT than with insecticides. The sterility method could be utilized as the final phase of an integrated programme or to prevent re-establishment from the peripheral zones.

Glossina populations may eventually develop resistance to insecticides. However, they should not become resistant to the effects of sterile males.

In areas where two Glossina species co-exist, the elimination of one species by the SIT is not expected to result in a sudden increase in numbers of the second species, since relative population size is not believed to be interdependent (provided the removal of one species is not followed by modification of the habitat which would make it more suitable for expansion of the remaining species).

Release of sterile males in low numbers can provide protection against a successful re-introduction of the species; present cost figures indicate that this technique might be cheaper and more effective than maintaining constant surveillance in an area already cleared but subject to reinfestation.

For example, the present cost of rearing tsetse flies approximates US $ 0.25/insect; aerial sprays of insecticides can be applied at no less than US $ 600/km^2. Assuming that two applications of an aerial spray over a 6-month period are necessary to prevent the re-infestation of a 1000 km^2 area cleared of tsetse, it would cost more to spray than to introduce 100 sterile male/km^2/month over the same time interval and area to prevent the re-establishment of infiltrating flies. The release rate/month approximates that utilized by Dame and Williamson to suppress a field population of G.m. morsitans with a growth rate that reached ca. 3.5x.

Where the control of tsetse in game reserves or national parks is regarded as necessary by the authorities concerned, the use of the SIT would minimize the detrimental side effects of chemical control. Reduction of the wild tsetse population may be necessary before sterile males can be effectively used. The use of not more than two applicatons of a non-persistant insecticide is most unlikely to cause any significant

contamination of the environment or damage to the fauna except where rare endemic insects are present. The benefit to visitors from elimination of tsetse should be balanced against these minimal side effects.

The SIT alone may be the method of choice where the disadvantages of chemical or other control methods, such as game elimination or bush clearance, must be avoided.

D. Disadvantages of the SIT

The method will not be practical for, nor is it designed to be used alone against, high natural populations. Under such circumstances, prior reduction by other means is a prerequisite.

When used alone, this method does not produce an immediate drastic reduction in insect numbers leading to a rapid interruption of disease transmission, as is the case with insecticides. This effect on disease transmission would be one disadvantage of using the method alone in areas where human trypanosomiasis is endemic and where immediate interruption of transmission is sought.

Release of large numbers of sterile flies in an area where trypanosomiasis is endemic could result in a temporary increase in the transmission rate, and release strategy should recognize this epizootiological problem. This would not normally occur if prior tsetse population reduction were accomplished, for even with the addition of sterile flies there would be far fewer flies than originally present. Nevertheless, studies are now in progress to reduce or eliminate possible transmission by released flies by feeding them prior to release on a suitable diet containing trypanocides or other chemical agents.

If there are several species of Glossina in the same area, control by the SIT will require an expansion of rearing if control of different species must take place simultaneously.

E. Additional research requirements

There is a great need for reliable data on tsetse ecology and population dynamics, especially on dispersal, that are directly related to the requirements for applying the SIT. These data are needed for different species of tsetse in different situations and different seasons.

Some species of Glossina have been successfully reared and sterilized in the laboratory, but continuing improvements in techniques would make the SIT more economical. For some species, the development of mass rearing methods is still required.

Techniques for the dispersal of large numbers of sterilized flies, either in the adult or pupal stage, should be improved.

It would be desirable to have more sensitive detection techniques to provide information on the presence and abundance of low-level populations. Detailed attention to all these points is presented in the following sections.

II. CHARACTERISTICS OF THE RELEASE SITE

It should be noted that the requirements of a release site and the characteristics of an area actually chosen for the release can be, and often will be, quite different.

A site for evaluating the SIT for Glossina control or eradication should ideally contain one species of Glossina and a homogeneous ecological condition and be naturally isolated from other Glossina populations but reasonably accessible. The area could be of real economic value following the elimination of the fly, yet should be sufficiently available and stable throughout the test so that research activities would not be unnecessarily complicated by premature development activities or local administrative policy. There should be a community of sufficient size available to offer suitable facilities for the construction and successful operation of a mass rearing complex and for adequate accommodation and amenities for the staff and their families.

In practice, an ideal field location for a release site rarely exists, particularly with respect to natural isolation. A field trial with the SIT could be located in any area that either meets or could be modified to approximate the requirements noted above.

A. Size of release area

A field trial has two primary goals: first, to test the ability of the sterile males to control a natural population, either with or without supplementary initial reduction measures; and secondly, to test the ability to maintain a sufficient production of flies to support the release programme. Thus, the treatment area should be large enough to encompass a variety of ecological conditions, so that the test will provide the broadest possible experience, and to ensure that host animal composition will not be disturbed. In addition, the treatment area should be large enough to require large numbers of sterile males and, thus, to demonstrate a practical rearing capability. The minimum size of a field plot meeting the above requirements should be about 1000 km^2 inside the barrier for a savannah species such as G. morsitans. A plot of 2500 km^2 would be more suitable; however, the research benefits derived may not warrant the increased costs. For riverine species, such as G. palpalis and G. tachinoides, a complete river system involving about 150 linear km of watercourse would constitute a meaningful size for release operations.

B. Isolation of the release site

If complete isolation is not naturally available, the creation of artificial barriers to fly movement would be required. For example, ruthless clearing, which is the complete removal of all trees and bushes from a zone of adequate width to prevent flies from crossing, could be used to create an artificial barrier. Alternatively, insecticide spraying could be used. The choice of insecticide, frequency of application and width of zone treated would be dependent upon the tsetse

species present and the character of the environment. Alternatively, a combination of clearing and insecticides could be used, as was the case in Tanga. Another effective and environmentally safe approach is the use of traps such as those devised by Challier and Laveissiere (1973) and Vale (Vale and Hargrove, 1979).

Under certain conditions, it may be economical to use sterile males to create a biological barrier around a central plot and to augment this with a perimeter treatment of a residual insecticide outside the biological barrier. A biological barrier could be used if the area was reasonably large; in this situation, flies could be released in a central plot (250 km^2) and additional releases would be made around the plot, extending about 8 km on each side. The total release would then encompass about 1000 km^2, but only the central plot would be directly evaluated.

The method of choice should effectively limit the immigration of fertile females into the release zone. Regardless of the type of barrier utilized, complete isolation may not be achieved unless the movement of pedestrians, cyclists, motor vehicles, domestic animals and game into the test area is very strictly controlled or, preferably, stopped, so as to prevent the introduction of tsetse. The cost of creating, maintaining and monitoring the effectiveness of barrier zones can be high in relation to the limited size of a test area and may form a fairly substantial component of the total cost of the trial.

C. Prior reduction of tsetse populations

The use of chemical or biological methods to achieve prior reduction of a naturally high population is a basic requirement. This would permit an increase in the size of the area that can be treated with a given number of sterile males and reduction of the frequency of release of sterile males. In other situations, the tsetse population may be so numerous that its control and elimination by the SIT could only be achieved by the release of excessive quantities of sterile males, whose production may not be feasible. Therefore, population reduction to a manageable level is required first. The use of pupal parasites would not be incompatible with the release of sterile adults.

The most practical method available to cause a rapid population reduction is insecticide spraying. A non-persistent compound would be necessary to prevent subsequent death of released flies. The dosage and frequency of application will depend upon the degree of reduction desired. The judicious use of non-residual aerial applications at the proper season may provide efficient reduction by eliminating most of the existing adult population with the first application and a large proportion of the adults that subsequently emerge from pupae with the second application. These reduction methods should be restricted to those habitats within the area that have unmanageable fly densities - perhaps as little as 10-20% of the entire area. Sterile male releases should commence with a minimum of delay after the last application, the length of this interval being determined by the time required for the breakdown of the insecticide used.

D. Recommendations

1. The release site must be isolated, preferably by natural barriers.
2. The effectiveness of the isolation should be monitored.
3. Tsetse fly populations must be reduced to levels where the SIT capability is feasible; a non-persistent insecticide applied discriminately may provide the most suitable method of population reduction.

III. ECOLOGY AND DISTRIBUTION

Geographical distribution of testse species

General distribution maps for species and subspecies are available for almost all countries situated in the tsetse zone.[1/] Unfortunately, it is only in areas where eradication operations are planned or in progress that accurate determinations of the distribution of each species have been made. This must be supplemented with careful and detailed studies in the area in which the SIT is to be applied.

Aerial and remote sensing photography can be extremely useful in determining tsetse habitats. Mosaics or print laydowns prepared on a scale of 1:30,000 or larger are most valuable in determining the distribution of tsetse habitats, in assessing the degree of isolation of infested localities and in planning infrastructural and logistic support facilities. However, the use of vegetative distinctions is of less significance in areas where some species of Glossina are less emphatically associated with a particular vegetative habitat.

The environment inhabited by the various species of Glossina is being constantly modified by human activity. This may result in alterations in the dominant species present, its distribution and the trypanosome infection rates in the flies. Examples of regressions and territorial expansions in tsetse populations have been frequently observed and, sometimes, have not been associated with intervention by man. In fact, some man-made alterations have merely succeeded in providing alternative habitats to the natural ones destroyed.

A. Local and seasonal variations in distribution

The success of the SIT depends in large measure on the relative number of sterile males to wild males competing for wild females. Thus, it is of utmost importance to be aware of local and seasonal fluctuations in population density in addition to the distribution of the species.

Population densities and distribution in most species, but particularly G. palpalis, G. fuscipes and G. tachinoides, especially toward the limit of their range, may become discontinuous with the general infestation as the habitat undergoes seasonal contraction and expansion. The same may be said of some temporarily isolated populations that may be continuous with the population of origin only for relatively brief periods, or in some years, not at all.

The ranges of tsetse flies in countries such as Uganda do not undergo the marked seasonal expansion and contraction that are sometimes displayed in countries with more extreme climates, hence the overall limits of distribution are reasonably constant. But with the savannah-inhabiting species, e.g. G. morsitans and G. pallidipes, local changes in density do occur.

1/ Available through IBAR/OAU Headquarters, PO Box 30786, Nairobi, Kenya.

B. Ecological problems

Before undertaking a field trial on the release of sterile males, the ecology of the species in the chosen area must be carefully investigated. Studies of the population dynamics of the fly should be made to elucidate the following points:

1. density-dependent and density-independent factors regulating population size;
2. estimation of the actual population densities as well as the seasonal dynamics of the population;
3. limits of the habitat during seasonal phases of population expansion and contraction;
4. tropisms and tropistic response in relation to movement of host animals;
5. estimation of seasonal survival of males and females;
6. variations and possibility of trypanosomiasis infection at different seasons and the time when flies can become infected;
7. determination of the dispersal of both males and females;
8. field application of current techniques; for example, the relationship between lipid reserves and residual blood meals and feeding intervals can be assessed to evaluate nutritional status as a measure of population stress;
9. use of pheromones and attractants to improve the accuracy of monitoring populations. Recent progress in the use of trapping devices suggests that our knowledge of populations should be drastically revised.

In addition, it will be necessary to determine whether populations of the same species or strains that are isolated either biologically or geographically differ in:

1. genetic incompatibilities;
2. sexual behaviour;
3. ecological preferences or niches that constitute an obstacle to mating or exchange of genetic material.

IV. METHODS OF MASS REARING

Techniques for tsetse colonization have progressed considerably and many of the problems encountered have been largely overcome.

The rearing of Glossina spp. using living animals as sources of food has been well-established at a number of institutions. All the tsetse species reared in laboratories in Europe and Africa belong to the morsitans or palpalis group. Currently, five species (G. morsitans morsitans, G. austeni, G. tachinoides, G. palpalis gambiensis, G. fuscipes fuscipes) are maintained at Maisons-Alfort, three species (G. m. morsitans, G. austeni, G. p. palpalis) at Langford, three species (G. m. morsitans, G. pallidipes, G. p. palpalis) at the IAEA Seibersdorf Laboratory, one species (G. p. palpalis) at Antwerp and one species (G. pallidipes) at Amsterdam (Table 2).

To test the feasibility of controlling tsetse flies using the sterile insect technique integrated with conventional methods, highly productive colonies of G. m. morsitans have been continuously maintained from 1975 to the present at the Tsetse Research Project, Tanga, Tanzania, and G. p. gambiensis has been raised at the I.E.M.V.T. Research Centre, Bobo Dioulasso, Upper Volta, from 1975 to the present.

Small colonies intended to produce limited numbers of flies for experimental purposes, e.g. physiological, genetic or irradiation studies, are maintained in various cities: Nairobi (ICIPE and ILRAD), Kenya; Achimota, Ghana; Lusaka, Zambia; Edmonton and Toronto, Canada; and Bonn and Hannover, Federal Republic of Germany, and Tororo, Uganda.

One of the major trends in research on tsetse rearing in recent years has been the development and use of membrane feeding techniques. During the past five years, studies at Langford and Seibersdorf on blood offered beneath artificial membranes have led to standardization of in vitro rearing techniques and have resulted in the establishment of large self-supporting colonies of G. m. morsitans, G. austeni and G. p. palpalis.

A. Environmental conditions

In general, all species tolerate temperatures between 24°C and 26°C, but show marked differences in their humidity requirements. Riverine species (palpalis group) prefer relative humidities of 75-90%, whereas savannah species (morsitans group) prefer lower relative humidities (60-70%). Puparia may be maintained at slightly lower temperatures and at somewhat higher relative humidity than for adult flies. For any given species, the environmental conditions should be maintained at the optimum level and variations should be minimized. Therefore, the use of reliable equipment for climatization is prerequisite.

Generally, all natural light is excluded from the rearing room. Lighting can be provided by a tungsten or fluorescent light directed onto the ceiling (tungsten is preferred), thus preventing direct light from

Table 2 Large-scale rearing of tsetse flies using living animals

Laboratory	Species	Origin	Start	Colony size (Mean)	Animal used (stock)	Puparia produced	Productivity (P = puparia)
EUROPE							
Tsetse Research Laboratory Langford, Bristol, UK	*G. austeni*	Tanzania	June 1963-April 1966	1977: 2,200	Goats	58,300	0.52 P/♀/week
I.E.M.V.T. Maisons-Alfort, France	*G. m. morsitans*	Zimbabwe	March 1965	1977: 4,006	Rabbits	74,036	1.54 P/♀/30 days
	G. tachinoides	Chad	April 1965	1977: 3,790	Rabbits	67,955	1.49 P/♀/30 days
	G. austeni	UK	November 1966	1977: 4,137	Rabbits	74,681	1.50 P/♀/30 days
	G. f. fuscipes	Central African Rep.	June 1968	1977: 3,052	Rabbits	47,083	1.03 P/♀/30 days
	G. p. gambiensis	Upper Volta	July 1972	1977: 3,985	Rabbits	73,191	1.34 P/♀/30 days
Laboratorium voor Oekologie Antwerp, Belgium	*G. p. palpalis*	Zaire	January 1971	1977: 4,000	Guinea pigs	76,320	1.59 P/♀/30 days
IAEA Seibersdorf Laboratory Seibersdorf, Austria	*G. p. palpalis*	Nigeria	June 1974	1977: 3,500	Guinea pigs	68,000	1.62 P/♀/30 days
AFRICA							
Tsetse Research Project Tanga, Tanzania	*G. m. morsitans*	Tanzania	November 1972	1977, 1978: up to 50,000	Goats and rabbits	Up to 100,000/month	Up to 6.5 P/♀/100 /day
I.E.M.V.T. - C.R.T.A. Bobo Dioulasso, Upper Volta	*G. p. gambiensis*	Upper Volta	March 1975	1977: 32,000	Rabbits and guinea pigs	58,000-60,000/month	To 1.72 P/♀/30 days

falling on the fly cages. The intensity of the light should be in the range of 1-20 lux at cage level. In order to reduce excessive fly activity, a 12-hour dl photoperiod is recommended.

B. Adult cages

Important elements in the design of cages for adult fly maintenance are: durability, cost, ease of manufacture, adaptability to feeding surface, ease of cleaning and the ease of stacking to simplify the collection of puparia. Many laboratories use modified Roubaud or Geigy-type cages. These consist of a stainless steel wire frame covered with non-absorbent terylene netting. The meshes allow the passage of deposited larvae and close contact of the adult fly with the feeding surface. The dimensions of the cages and number of females per cage are variable. Most cages in use are ca. 15 x 8.5 x 5 cm or ca. 25.5 x 12.5 x 3 cm and hold 10 to 30 mated females. Also commonly used are round or oval cages of polyvinyl chloride (PVC) (Mews et al, 1972). Standard cages (12.5 cm in diameter and 4.5 cm high) may hold 15-20 female flies and the larger ones (made from ca. 25 cm diameter tubing) may hold 50 flies. The flies are added to or removed from the cage via a hole cut in the side which is closed with a cork. These cages have tightly-stretched terylene netting on two surfaces and, therefore, are very suitable for membrane feeding.

Recently, a high degree of efficiency was obtained by maintaining 20-30,000 G. p. palpalis females in groups of 250 in 10-liter plastic containers covered with terylene netting (Figure 1).

C. Maintenance and handling methods

Many of the handling techniques and devices employed in maintaining colonies of tsetse flies at various plants are very similar. These techniques, described in a number of publications (Azevedo et al, 1968; Evens and Van der Vloedt, 1970; Geigy, 1948; Itard et al, 1968; Mews, 1970; Mews et al, 1976; and Nash et al, 1966, 1968) were reviewed and summarized by Mulligan (1970) and Laird (1977). Efficient methods have been developed for the collection of newly emerged flies, separation of the sexes by chilling in cold air at 2-4°C after mating and collection and storage of puparia. The possibility of immobilizing flies using pure nitrogen gas has also been tested. Handling techniques associated with the maintenance of tsetse colonies by artificial feeding methods or by the use of living animals are essentially the same. The only differences are related to the method of presentation of the food and techniques to ensure optimum food intake by the flies.

One of the factors influencing the productivity of a tsetse colony is mating. Both the sexual maturity of the flies and the mating technique are of paramount importance. It has been found that mating can be carried out most efficiently by introducing an excess of males over 8 days old into a cage containing 2-3 days old females and leaving the sexes together for 24-48 hours.

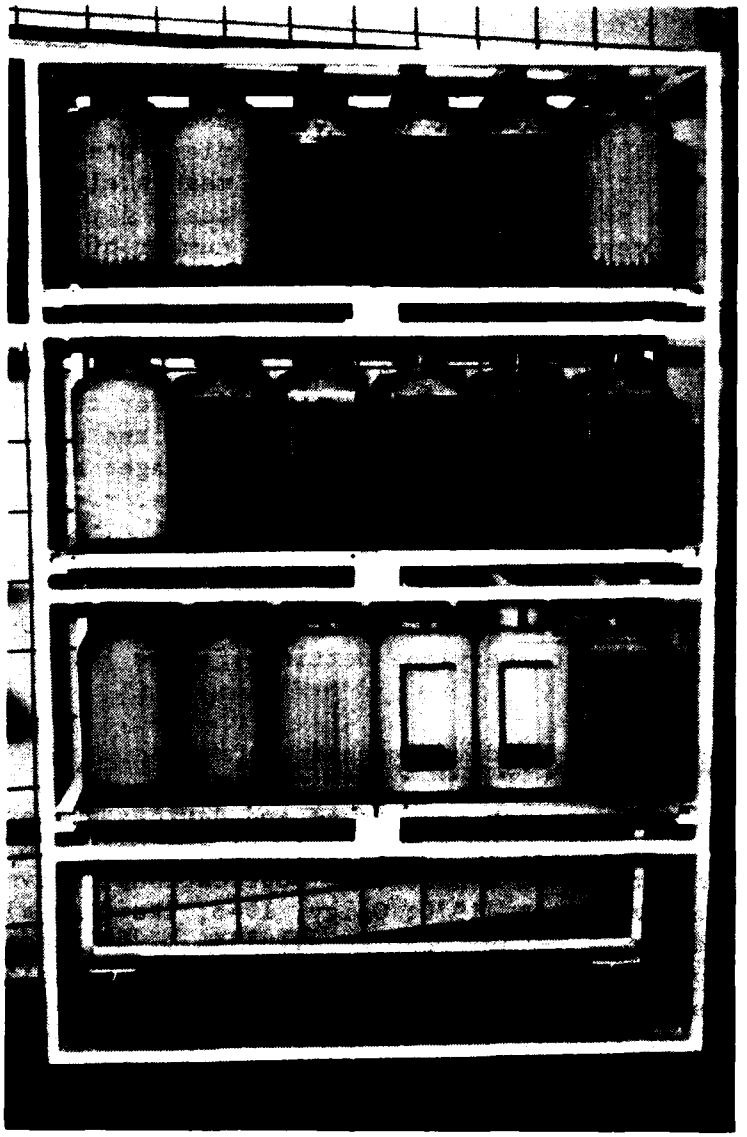

Figure 1

Technique for mass rearing Glossina palpalis palpalis using large (10-litre) plastic cages covered with terrylene netting. Each of the cages shown above can hold up to 250 flies.

Individual male tsetse flies are capable of successfully inseminating several females (Jordan, 1972). Thus, for small studies, males previously used can be retained as stud males for up to six matings.

D. Feeding methods for colonization

1. Feeding on living animals

Cattle, goats, rabbits and guinea pigs can all be used for the feeding and maintenance of adults in laboratory colonies. The type of animal used varies with the facilities available and individual preference. However, the following criteria should be used to evaluate and choose the most suitable host:

1. the overall performance of the host animal herds in terms of survival, productivity and quality of the offspring;
2. the cost of obtaining, maintaining and breeding;
3. disease problems associated with the various animal hosts;
4. the ease and economy of handling in application of cages to the host animal, and the time required to complete the feeding process. Consideration should be given to the number of flies that can be fed per unit time and the cost of labour.

Rabbits, goats and guinea pigs are the most promising of the hosts that have been used in large-scale rearing. At Langford, lop-eared rabbits were the preferred host for G. morsitans and are also being used for mass rearing of G. tachinoides at the I.E.M.V.T. Bobo Dioulasso project. However, rabbits have serious drawbacks in that they are quite susceptible to respiratory infection. Goats are considered by the Longford group to be the ideal host because of their hardiness, ease of handling, large size, which allows for the feeding of several hundred flies per day; good reproduction rate, and widespread availability. They were also used successfully for mass rearing G. m. morsitans at the Tanga Tsetse Research Project.

The feasibility and practicability of using guinea pigs for tsetse rearing has also been recognised. They have a high reproductive potential and are easy to handle. They have been extensively used for rearing G. p. palpalis at Antwerp and Seibersdorf and have recently been introduced as an alternative host for G. p. gambiensis at the I.E.M.V.T. Bobo Dioulasso Project. They have also been used successfully for many years in Salisbury, Zimbabwe, for feeding G. morsitans and G. pallidipes.

2. Artificial methods of feeding (in vitro)

In vitro feeding methods are now in use at several research centres. The flies obtain blood by piercing an artificial membrane. Approximately 100 ml of blood are needed daily to feed up to 1500 flies on a 45 x 45 cm surface. Feeding tsetse on fresh blood using the membrane technique has become a matter of routine at the Seibersdorf and Langford Laboratories (Mews et al, 1976). The advantage of not having to keep host animals is to some extent offset by the need for a regular supply of fresh blood. However, it was demonstrated that haemolysed

blood, stored by deep freezing, could be used. Since the flies do not need intact erythrocytes for their nutrition, they can also be maintained by feeding on freeze-dried, reconstituted bovine blood. This powdered blood can be stored over a period of six months and is reconstituted by adding distilled water equal in volume to the amount lost during the evaporation process (Figure 2) (see Wetzel, 1980).

E. Animal Housing and Management

Since animal housing and management are of prime importance in rearing facilities, the following aspects should be taken into consideration:

1. animals should be confined indoors continuously while used for feeding tsetse;
2. floor pens and caging equipment within the animal house should be arranged to allow easy access to all animals for both observation and handling;
3. attention should be given to an environmental control system to keep temperature and humidity variations within an acceptable range;
4. a balanced diet is essential. All material and foodstuffs should be screened for insecticidal or pharmaceutical content in order to prevent the presence of toxic compounds in the hosts;
5. obnoxious odors, accumulation of excreta and occurrence of ectoparasites can be minimised with proper sanitation of pens and cages;
6. host animals treated for disease or parasites should be isolated; an adequate quarantine period must be allowed before the animals are returned to the holding area and used for fly rearing;
7. the effects of the feeding pressure on the host animals should be carefully monitored.

F. Mass rearing facilities

The current status of semi-large-scale and large-scale colonization of tsetse flies using living animals is summarised in Table 3. Highlights of fly rearing activities during recent years include the mass rearing of G. m. morsitans at Tanga, Tanzania, G. p. gambiensis in Bobo Dioulasso, Upper Volta, and G. p. palpalis in Vom, Nigeria (BICOT).

During the 1977-1978 period, the Tanga Project reached its full project fly capacity of 60,000 flies distributed over four separate insectaries that produced ca. 100,000 pupae per month. Flies were fed primarily on goats. The most efficient of the four insectary units contained 25,000 flies (about 4,000 males and 21,000 females) with both goats and rabbits as hosts. A trained cadre of rearing staff and refined fly colony and host animal management procedures resulted in stabilised colony performance that allowed for a constant removal of ca. 48% of the pupal production for releases.

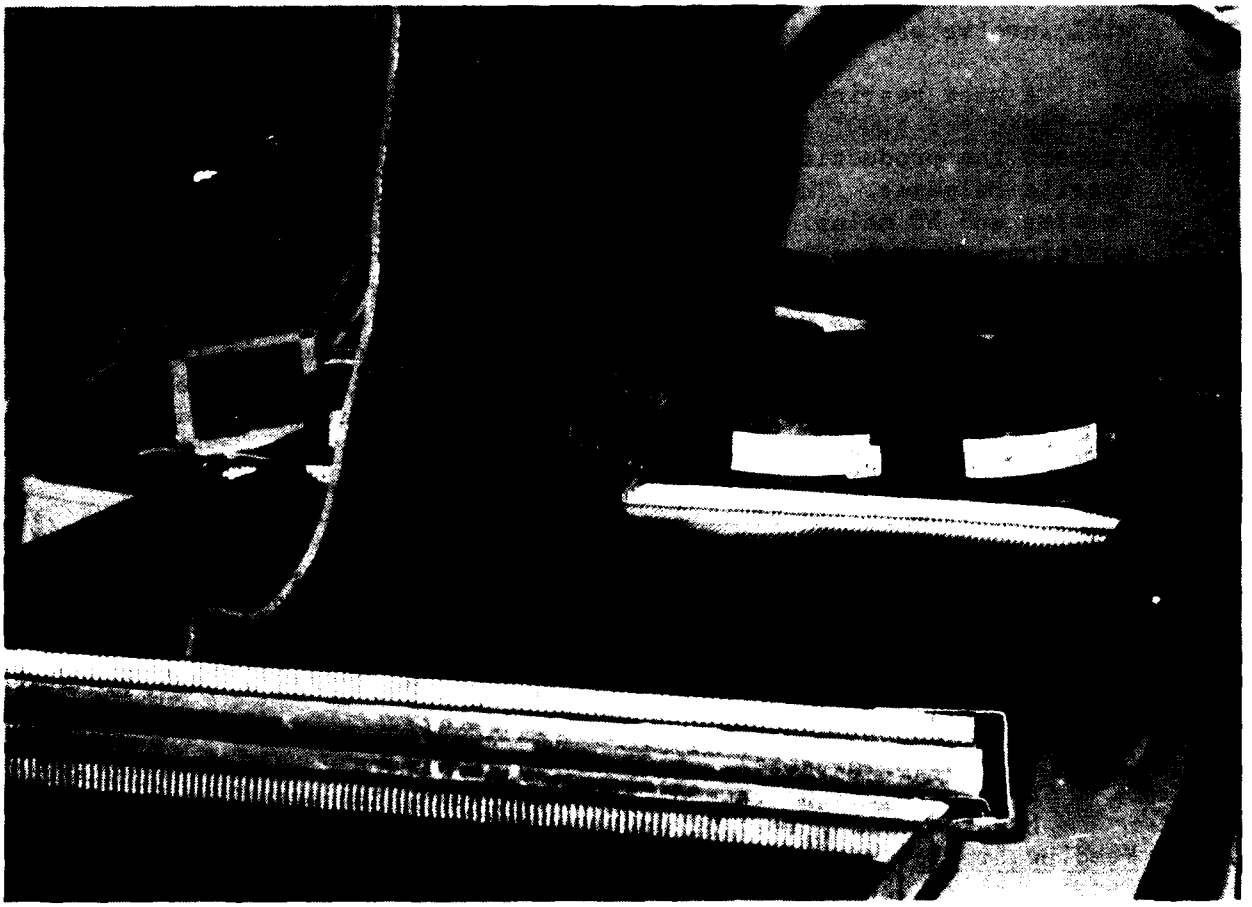

Figure 2

Technique for feeding tsetse flies in vitro, using an electrically warmed plate, reconstituted freeze-dried blood on a grooved aluminium plate over-laid by a silicone membrane.

At Bobo Dioulasso, G. p. gambiensis is currently reared in two separate insectaries. The main stock colony is kept at a level of ca. 32,000 females and fed on rabbits. A second unit, initiated in April, 1977, and containing between 10,000 and 15,000 flies, is fed on guinea pigs. During 1977, the entire colony, containing about 36,500 females, produced over 710,000 puparia.

The creation of separate mass rearing units, such as at Tanga and Bobo Dioulasso, provides an insurance against loss of the entire breeding stock from accidents. The mass rearing approaches contemplated for BICOT will involve both in vivo and in vitro feeding methods.

A mass rearing system using guinea pigs as a food source has been developed for BICOT at the Seibersdorf Laboratory and is designed to support the production of 3,000 or more G. p. palpalis males per day for sterile releases. The system consists of 10-liter cages holding 200 females and 80 males. Two rows of six cages, each three tiers high, are positioned on a trolley (Figure 3) occupying about 0.5 m^2 of floor area. On the assumption that 0.02 of the female population approximates the number of males available for release, the females caged on one trolley can produce over 150 males per day for release in the present system. Using this approach, only 14 m^2 of holding area are necessary to hold 100,000 females. Moreover, handling is reduced since the flies can be fed in the holding room.

1. In vivo feeding

The requirements for using the previously described method for mass rearing of a G. p. palpalis colony consisting of 100,000 females and 30,000 males include cages, feeding trolleys, guinea pigs and a minimum number of technicians to man operations (Figure 3). Estimations are as follows:

Cages (10-litre, plastic): 650, of which 500 would hold females. At an average stocking rate of flies per cage, the actual number of cages handled during feeding would be 500.
Feeding trolleys: 12, each holding 50 to 60 cages.
Feeding racks (shipple type): 200, each to carry 4 guinea pigs (Figure 3)
Holding trolleys: 25, to accomodate 16 cages of 250-300 flies each.
Guinea pigs: 200 will be required as breeding stock and 1000 for fly feeding. Approximately 600 guinea pigs will be used daily for feeding 130,000 flies.
Technicians: 6 (one technician/2 trolleys) and 2 as a stand-by for weekend and holiday work. Three additional technicians will be required for operations in the animal house.

Additional criteria under evaluation are as follows:

1. The composition of the female population in the colony. It should be approximately 35% flies 20 days or younger, with a mean daily mortality of 0.5%; 40% flies between the ages of 20 and 50 days, with a mean daily mortality of 1.2%; and 25% flies between the ages of 50 and 80 days, with a mean daily mortality of 2.0%.
2. The male population should be about 1/3 as large as the female population, with 70% of its number being between 5 and 25 days of age.

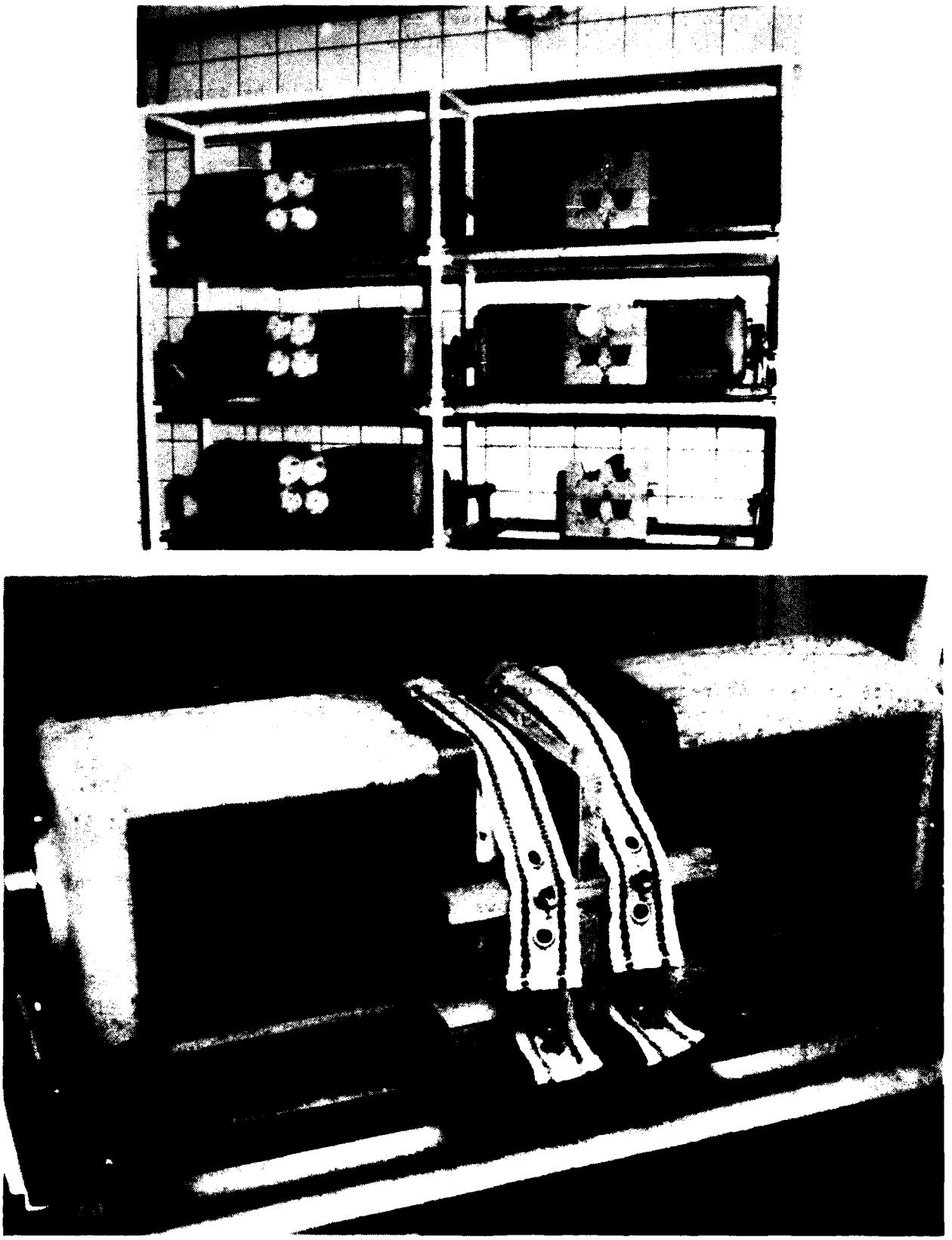

Figure 3

Feeding tsetse flies on guinea-pigs. The arrangement shown here allows the feeding of 500 flies on 4 guinea-pigs within 5 minutes.

3. The mass production method should involve a minimum of 250 flies per cage, with productivity assessed on a unit, rather than an individual, fly basis (1 cage = 1 unit).

Under these conditions, actual feeding time would be approximately 5 hours per technician per day, plus a total of 3 hours of rest and break periods. Thus, the entire operation involving the use of 600 guinea pigs and 6 technicians to feed 130,000 flies held in 500 cages would take 8 hours per technician per day. At the indicated rate of feeding, fly challenge would be 170 per guinea pig per day.

Operational costs

Exclusive of personnel, the estimated cost of rearing 100,000 G. p. palpalis in vivo would be US $ 25,000 per year. Details are as follows:

Purchase and maintenance of guinea pigs	$ 15,000
Cost of 650 fly cages	3,000
Cost of trolleys (12 holding and 25 feeding)	6,000
Cost of 200 feeding racks	1,000
	$ 25,000 *

*cost/released pupa exclusive of personnel and facilities ca. US $ 0.03

2. In vitro feeding

A truly mass rearing system using in vitro feeding is yet to be developed. Cages currently used for holding flies fed in vitro have a capacity of up to 20 flies (as compared to 250 per cage in the in vivo system). Therefore, a major requirement for mass rearing in vitro is the development of fly cages that would hold at least 250 flies and, at the same time, permit easy application to the membrane surface to enable flies to feed readily.

The system currently in use at Langford feeds 500 flies on 50 ml of blood, while that at Seibersdorf allows the feeding of up to 2,500 flies per membrane (100 ml blood). It is estimated that, at this rate, a colony of 100,000 females and 30,000 males would require about 50 membranes, 6.5 litres of blood daily and 5 technicians for the operation, which could be completed in about five hours. The present level of in vitro feeding operations at Seibersdorf can be easily improved and could, therefore, form the basis for the development of a mass rearing system.

3. Location of rearing facility

The rearing facility should be located within or in close proximity to the release zone and at a place where adequate electrical power and water are available. It should be in or near a community where good transportation and commercial services exist. A major precaution in the placement of the rearing facility is the avoidance of local sources of insecticide contamination.

4. Initiation of colonies and precautions

Whenever possible, colonies should be initiated from collections of wild puparia. Flies collected from the field may harbour trypanosomes and should be kept in special cages to ensure the protection of the staff against accidental infection. The flies should be kept separate from the colony flies and, if fed in vivo, fed on a separate group of animals.

Footwear is a known source of insecticde contamination in insectaries, particularly visitors who may have toured cattle dipping operations before reaching the tsetse rearing facility. Insect sprays contaminate clothing as well. Entry of persons not directly concerned with the fly-breeding units should be restricted and when entering the plant should wear laboratory coats and special slippers provided. Access points to the building should be limited. Laboratory staff should be attired in special clothing free of contamination.

A small laboratory should be included in each rearing unit where pupae can be weighed and investigations or quality assays carried out.

Emergency power plants should be provided to guard against interruption in electrical supply.

Provision should be made for supervision of the rearing facility thoughout the day and night. To simplify the day-to-day operation of the units, a 7-day work week is desirable. This would necessitate employment of an adequate staff to allow for weekend and holiday work, as well as for emergencies such as illness of staff members.

6. Recording results

A variety of methods of recording results have been employed in the past, which makes comparison of results between laboratories rather difficult. It is recommended that two types of records be maintained in all laboratories:

1. Colony data. Daily records of the additions and subtractions to the colony, plus the number of puparia produced (Tables 3 and 4).
2. Group data. Records of fecundity, longevity, puparial weight, etc. (Table 5).

H. Mathematical models

One of the prime requisites in the SIT to control and/or eradicate pests is the availability of a facility and mass rearing technology to produce the number of insects necessary to carry out the programme. Moreover, the capacity of producing the insects at the lowest possible cost is of utmost importance. Basic procedures for the mass rearing of the tsetse have been developed, but the cost/unit is still relatively high. In-depth analysis of all rearing parameters, such as fly adult cut-off time, productivity as opposed to numbers of flies per cage, and man-hours necessary; must be conducted to determine the proper balance to

P L	EXP.:	IAEA TSETSE RECORD SHEET
Fec lx		DATE COMMENCED:
Pw		DATE TERMINATED:

CAGE No	AGP 1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	TOTAL

N 59(Dec 72)

Table 3

IAEA TSETSE RECORD SHEET

COLONY: **TREATMENT:**

DATE COMMENCED:
DATE TERMINATED:

MONTH	EMERGED		♀♀ PRE-PRODUCTIVE			PRODUCING ♀♀				Σ♀♀	PUPAE	PUPAL WEIGHT	
	♀♀	♂♂	INPUT	DIED	TOTAL	INPUT	DIED	OUTPUT	TOTAL			TOTAL	MEAN
1													
2													
3													
4													
5													
6													
7													
8													
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28													
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30													
31													
Σ													

Table 4

Table 5 Performance of G. p. palpalis caged per 40 and fed six days a week on guinea pigs. Feeding time: 5 min. (Eight guinea pigs per 1000 flies)

COLONY: GLOSSINA PALPALIS PALPALIS (Kaduna)

D. ECL DECEMBER 1970						D. MAT. DS 2000/1000						D. SEP. After 5 days				No.F. 1000	
DATE	♦	N°♀♀	N° PUPAE	Ab.	WEIGHT		DATE	♦	N°♀♀	N° PUPAE	Ab.	WEIGHT					
		1000						3	889	43	1						
								6	883	42							
								4	879	63	4						
		1000						2	877	72							
	2	998						5	872	70	2						
	5	993						9	863	110	3						
	2	991						7	856	47	1						
								7	849	73	5						
								6	845	33	1						
10	2	989					50	5	840	63	2						
Σ							Σ			685	28	2821					
	1	988						3	837	37	2						
								5	832	72	3						
								12	820	52	2						
	1	987						8	812	47	1						
	3	984						6	806	35	4						
	1	983						10	796	39	5						
	2	981	70	1				1	795	53	2						
	1	980	89					8	787	77	1						
	1	979	182					11	776	87	2						
20	5	974	149	4			60	22	758	68	4						
Σ			700				Σ			606							
	5	969	56	1				5	753	32	1						
	4	965	27	1				8	752	26	3						
	1	964	35					20	736	54	3						
	2	963	32	2				3	731	14							
	6	957	57					16	707	68	2						
	1	956	103	1				7	700	57	5						
	3	953	37	3				9	691	88	2						
	1	952	111					16	673	44	2						
	4	948	43					11	662	86	1						
30	2	946	62	2			70	11	649	43	2						
Σ			747	23	2447		Σ			606	23	3121					
	5	941	29					28	632	32							
	3	938	43					11	618	36	2						
	2	936	52					20	586	28	3						
	5	930	59	3				8	580	33	3						
	4	926	133	3				12	577	24	2						
	5	921	72	2				5	572	38							
	3	920	30	3				24	546	28	3						
	9	911	109	3				27	513	50	3						
	11	891	19	3				15	501	13	3						
40	4	887	53				80	12	488	13	1						
Σ			892	23	3139		Σ			318	23	3457					

N-88 (May 75)

Table 6 Productivity and availability of surplus males from a colony [a] of G. p. palpalis held for the indicated number of days. Production based on a colony of 100,000 females and 25,000 males having a sex ratio of 4.5 M:5.5 F at emergence. An adult eclosion rate of 95% and a daily 0.3% mortality is assumed.

Terminal age (days)	80		70		60		50	
puparia/ initial ♀	4.6	4.2	4.2	3.8	3.6	3.3	2.9	2.7
initial puparia/day	5750	5250	6000	5420	6000	5500	5800	5400
no. flies emerging	5462	4988	5700	5149	5700	5225	5510	5130
daily production:								
females	3004	2743	3135	2832	3135	2874	3030	2822
males	2458	2245	2565	2317	2565	2351	2480	2308
no. males required for colony	312	312	357	357	417	417	500	500
no. avail. for release per day	2146	1933	2208	1960	2148	1934	1980	1808

[a] A colony of 100,000 females should produce approximately 2,000 males per day for releases. If the treatment area is 1,000 km^2, an average of 2 males per day would be available per km. If survival averages 50 days, a continuous supply of males should be available to release 100 per km^2. However, if a 4:1 ratio is desired, the wild population of males to be challenged should not exceed 25 per km^2.

achieve maximum output for minimal cost. Enough mass rearing parameters are now available to derive models to assess colony productivity (Jordan and Curtis 1968; Curtis and Jordan 1970 for G. austeni and Jordan and Curtis 1972 for G. m. morsitans). Studies have been initiated in this area, varying from a preliminary mathematical model by Fried (1980) as described in Table 6 to a preliminary computer model by Timischl (1977).

Recommendations

1. Research for mass production techniques should aim at developing a system for holding a minimum of 250 flies per cage and productivity assessed on a unit basis rather than on individual flies
2. Handling of individual flies should be eliminated.
3. Experiments to determine the optimum type and size of cage and number of flies per cage for the particular rearing method should be carried out with the objective of reducing costs and handling of flies.
4. Separate tsetse rearing units should be used rather than one large unit. Each unit should include its own staff, host animal housing and fly colony.
5. When host animals are used, their breeding and maintenance should be supervised by qualified personnel.
6. Stringent precautions should be taken to avoid insecticidal contamination.
7. Research on membrane methods of feeding should be given a high priority.
8. Quality controls applicable to mass rearing in Africa must be developed.
9. Computer programs (for hand or desktop computers) for colony performance should be derived.
10. A sexing method other than chilling should be developed.
11. Synchronisation of adult emergence should be investigated.

V. STERILIZATION PROCEDURES

For the SIT to be effective, the sterilization procedure must result in a male with low fertility, high survival and maximum competitiveness.

A. Sterilization of adults

Tsetse flies can be permanently sterilized with gamma radiation or with chemosterilants. The dose of gamma rays necessary to induce total sterility in adult male G. morsitans is 25 krad (Itard, 1969). As male fertility is reduced with increasing radiation dose, survival is also reduced; at a dose of 25 krad, overall survival is less than 50% that of unirradiated controls (Itard, 1969).

Several species of tsetse flies have been investigated to determine the effects produced by various doses of gamma radiation. Doses that induce 95% sterility are shown in Table 7.

Table 7. Dose of gamma radiation necessary to produce ca. 95% sterility in 5 species of male tsetse flies (outcrossed with normal females) when irradiated at the indicated stage of development.

Species	Stage treated	Dose, krad	Approx. survival(% of control)	Reference
G. austeni	9-day adult	8-9	84	Curtis, 1968
G. tachinoides	1-9 day adult	10	37	Itard, 1960
G. pallidipes	1-3 day adult	16	74	Dean and Clements, 1969
G. morsitans	0-1 day adult	12	-	Mews (unpubl.)
	0-4 day adult	17	50	Itard, 1969
	9 day adult	13	-	Curtis (unpubl.)
	puparium: 1-7 days before emergence	9-12	50	Dean and Wortham, 1959
	puparium: day of emergence	10	80	Curtis and Langley 1972
G. p. gambiensis	1-3 day adult	10-11	37	Taze et al, 1977

Total male sterility can be achieved in 1- and 2-day old adult G. morsitans by contact with the chemosterilants tepa or metapa and survival is not reduced in laboratory trials by exposures of 30 or 60 minutes to residues of 10 mg/ft^2 on glass. In two separate release trials conducted over a period of 25 months (1967-69), principally with males treated when 1-day old, the overall sterility achieved in released males was 99.8% and the survival of treated males in the laboratory after 34-40 days was 77%, equal to that of untreated males after 34 days (Dame and Birkenmeyer, unpublished data).

B. Sterilization of pupae

A high degree of sterility (95%) is obtained by exposing G. morsitans pupae of mixed ages to doses of 8-15 krad gamma rays, although all pupae that have not completed one-third development are killed by these treatments (Dean and Wortham, 1969)..

Work on G. morsitans pupae of known age (Langley, Curtis and Brady, 1974, unpublished data) shows that pupal viability is 100% following exposure to 10 krad during the final one-third of development. Pupal viability is not significantly reduced following a dose of 15 krad but falls to 80% of normal with 20 krad and a minimum of 50% with 25 krad and above. This mortality is due entirely to the death of male pupae, thus confirming the observation of Dean et al (1969) that male pupae are more radiosensitive than female pupae in respect to survival. At 25 and 30 krad, virtually no male pupae survive. Work on G. austeni pupae shows that pupal viability is optimal (more than 80% of control) following 10 krad only if pupae are exposed during the final one-third of development (Langley and Abasa, 1970). Pupal viability at this stage of development is not reduced by doses up to 8 krad, but then falls to 80% at 10 krad and to a minimum of 50% at 20 krad and above.

Based on present knowledge, it appears that pupal irradiation must be confined to the last one-third of the developmental period and that doses no higher than 15 krad can be used if a maximum of male pupae are to survive. Such a dose should produce more than 95% sterility in the emergent males (Table 7).

It has long been known that irradiation in the absence of oxygen reduces both the genetic and somatic damage. G. morsitans pupae have been irradiated in atmospheres of nitrogen or air. These experiments were carried out with treatments given to male pupae on the last day of the pupal period. It was found that male survival for a given level of fertility reduction was higher from pupae irradiated in nitrogen than from pupae irradiated in air (Curtis and Langley, 1972). Computer predictions suggest that 17 krad in nitrogen which produces a residual fertility of 7% but a survival of 95% will lead to extinction of a population in 7 generations with releases of only 22 sterile males per 100 wild males. However, 18 krad in air which produces only 0.5% residual fertility will be ineffective at this release rate owing to depressed survival (Curtis and Langley 1977).

Irradiation in air on the last day of the pupal period induces immediate emergence (Curtis, 1970). Nitrogen temporarily inhibits this

effect and the stimulated emergence can be further delayed by subsequent maintenance for up to 3 hours at 11°C in air. Such a procedure would give time for aerial distribution of the chilled pupae and almost all of them would emerge within one hour of returning them to normal temperature at the release point (Curtis and Langley, 1972).

C. Effects on survival and competitiveness

Radiation sterilization of pupae and chemosterilization of adults did not appear to affect the competitiveness of male G. morsitans when tested under laboratory conditions and in field cages up to 288 ft^2 in area, (Itard, 1970; Dean et al, 1969). However, the competitiveness of adults exposed to 8 or 12 krad when 1 day old was reduced to a level where it would require 2 sterile males to compete equally with 1 untreated male. More recently, analysis of behavioural responses of males suggests that there is a correlation between survival and sexual comptetiveness of G. morsitans irradiated as pupae (Langley, Curtis and Brady, 1974).

D. Chemosterilization vs. radiation

For release of flies treated in the pupal stage, gamma radiation has the advantage of safety, accuracy and predictable effect on the treated insects, when compared with chemosterilants. Also, doses that produce a high degree of sterility in males always result in total female sterility. Reduction in adult survival following pupal irradiation can be minimized by using nitrogen. Adult treatment with chemosterilants offers an advantage because, under carefully controlled condition, total permanent sterility can be achieved; survival equals that following radiation treatments in air that induce 95% sterility. Available information, though limited, suggests that the competitiveness of males irradiated as pupae with 8, 12 or 15 krad in air or treated as adults with tepa, either by contact or spray application, is superior to that of adults sterilized with the same doses of gamma rays (Dean et al, 1969).

In an eradication programme where large numbers of pupae of known age are available, chemosterilants do not appear to offer any advantage over radiation sterilization except the capability of producing complete sterility with less effect on survival. With field-collected pupae of unknown age, chemosterilants provide a means of inducing sterility in emergent adults without the high mortality associated with gamma-irradiation of pupae that have not completed two-thirds development. From a statistical viewpoint, it is unlikely that 100% sterility need be attained in released males. If full sterility is deemed necessary, it is possible that a combination of chemosterilant and radiation treatments of pupae can be developed.

Sterilization of adult males and subsequent release would permit sexing and the return of the females to the breeding colony. This would allow a 35% increase in output from a colony of G. morsitans compared with the sterilization of unsexed pupae. In addition, it would protect males from natural mortality before the age of maximum mating potency. However, this would add appreciably to the requirement for fly breeding

since the male tsetse flies would require feeding. In addition, it may result in the liberation of flies with considerable wing damage as well as inhibition of flight muscle development caused by confinement in cages.

E. Optimum level of sterility

In sterile insect projects against oviparous insects, very high levels of sterility have been used because it is thought that populations of such insects are strongly buffered by density-dependent factors; if a population could increase 10-fold per generation in response to a reduction in density, and if there was 10% residual fertility in the released males, the population could not be driven to extinction, however many males were released. Though there is no firm information for tsetse flies, it is unlikely that artificially reducing a population could cause it to increase subsequently by more than two-fold per generation. On this basis, it can be shown that there would be a considerable advantage in using nitrogen during irradiation. A very high degree of radiation-induced sterility would be counter-productive because of the correlated decline in survival (Curtis and Langley, 1972). This would be even more true where the decline in survival was correlated with a decline in competitiveness.

The above estimates assumed that F_1 progeny of irradiated males were all fully fertile. However, low radiation doses in G. tachinoides, G. morsitans and G. austeni produce a considerable proportion of F_1 sterility and semi-sterility (Curtis, 1969). This reinforces the view that radiation doses that give considerably less than 100% dominant lethality may have a better chance of achieving eradication of a tsetse fly population than those irradiated at higher doses.

F. Other genetic approaches

The elimination of large populations of tsetse flies using genetic mechanisms other than dominant lethals requires, primarily, a fundamental knowledge of natural and induced variation of those genetic systems involved with the reproductive biology of the species. The potential of genetic methods of control, some of which are outlined below, may be realized when working details of the following parameters are known:

1. Vanderplank (1948) Curtis (1972) and Curtis, Langley and Trewern (1980) showed that matings between various Glossina subspecies were possible but were sterile or produced sterile hybrids. Partially sterile mutants of G. austeni have also been produced (Curtis 1969). The release of males of a suitable 'foreign' form would be equivalent to the release of sterile males, provided that there were no barriers to mating with the local form. This technique would remove the necessity for any sterilizing treatment and, hence, avoid the somatic damage that this may cause. The use of genetically incompatible strains requires absolute accuracy in sexing prior to release, so that only one sex is released.

2. One potentially useful system involves the x-linked mutant salmon (Gooding, 1979). Release of salmon males into a population would reduce the total number of flies due to the maternally influenced lethality in progeny of homozygous females. At the same time, heterozygous females would act as carriers for the allele in the population. The Hardy-Weinberg equilibrium realized would depend on the initial ratio of mutants to wild type males. "A simplified model of a tsetse population which assumes 8 generations per year, a maximum reproductive rate of 50% per generation, and an annual cycling of the population of five-fold has been created. Assuming that, at the beginning of each generation, fully competitive salmon males could be released into the population at a rate of 3x the annual minimum number (= 60% of the annual maximum), it can be shown that within 2 years the field population would be reduced to 0.01% of the original density" (Gooding, 1979).
3. Similarly, the use of phenotypic markers (colour or electrophoretic) could facilitate the identification of sterile males in an SIT. Migration in and out of control test areas is similarly amenable to close monitoring with such genetic markers.
4. Data on racial differences and incompatibilities would be useful for the assessment of the genetic isolation mechanisms operating in the field and potentially useful for the SIT.
5. A search for genetic resistance to trypanosomes and the elucidation of its chromosomal location should be undertaken for its eventual introduction to wild populations. It is not envisaged that genetic mechanisms are as yet feasible to drive a refractory gene through a population. Introduction would be dependent on a sustained release.

Recommendations

Studies on the following points should be undertaken:

1. field evaluation of sterilized insects, including an assessment of competitiveness by capturing inseminated females and measuring their fertility;
2. determination of the extent to which sterility occurs in progeny from irradiated males and the benefits accruing to a sterile release project from this phenomenon;
3. evaluation of the effects of manipulating the pupal holding environment to synchronize pupal development for sterilization and release logistics. Pupae should be collected at least daily;
4. evaluation of the effects of irradiation on the vectorial capacity;
5. effect of radiation on fly symbionts;
6. research with new and safer chemosterilants should be continued.
7. combination of effective attractants/arrestants with chemosterilization procedures should be attempted in order to obviate the necessity of rearing flies for sterilization and release.

VI. DISTRIBUTION METHODS FOR STERILE MALES

The expense of rearing tsetse pupae under ideal conditions is such that losses after production must be minimized. The distribution of sterile males in field programmes may entail shipment, storage and release of either pupae or adult flies. The pupae or flies may be taken from the rearing facility directly to the release site or to a reception centre for further holding prior to release.

A. Adult shipment

Adults can be housed in cages placed in thermal boxes. The dimensions should be suitable for manual transport. One box currently in use holds two 14 x 8 x 5 cm cages; this permits the transport of 75-150 flies. The flies could be fed prior to shipment and then maintained in the thermal boxes at 4-10°C by using dry ice. Depending on the distance to and the conditions at the reception site, one can ship flies by air, truck or bicycle. If shipment is by air, a night shipment might be preferable; if by truck, it would be useful to have the carrying compartment fully air-conditioned and have a separate suspension to reduce fly disturbance caused by uneven motion of the vehicle. Care should be taken not to chill flies immediately after feeding as this can be lethal.

B. Pupal shipment

The transportation of pupae is simpler than that of adults, as they can be shipped in large numbers yet occupy little space. Pupae can be immobilized and protected against injury from vibration by utilizing cotton or wood or plastic shavings. The temperature should be between 4-10°C to prevent eclosion, but higher temperatures are acceptable if eclosion is not imminent. Shipment conditions are not as stringent as those required for adults, but pupal survival is much better when they are shipped after completion of the first half of the pupal period.

C. Storage

The reception of an air shipment should take place at the landing site, if this is possible. In other cases, shipments could be parachuted to a prearranged site and received by the reception team. The delivery truck or other ground transport does not present any particular difficulty if 4-wheel drive vehicles are used.

The receipt of adults or pupae at the storage site must be tended by a conscientious team. The flies should be immediately placed in a suitable holding environment and be maintained there until redistributed for release; care should be taken not to overcrowd flies in the holding containers as this could have detrimental effects on fly quality. If they are maintained continuously at 4-10°C, the interval between shipment and release of adults should not exceed 24 hours.

Considering the numerous possible delays that could occur in the shipment of adults and pupae from the point of production to the point of release, it may be necessary to establish secondary reception centres where flies could be received, fed or stocked, and reshipped. If necessary, sterilized pupae could be maintained until eclosion at these centres. This would permit the isolation of males from females, since the release of females is not necessary.

D. Releases

Whatever conditions of shipment and storage are utilised, the final action will involve the release of sterile males in the environment. Releases from the ground are theoretically preferable to aerial releases because of the ability to place the released flies in the proper environment and, thus, reduce losses. For this purpose, releases could be made from fixed ground stations, which might be reached by foot, bicycle, automobile or fixed or rotary-wing aircraft.

Under certain circumstances, such as for inaccessible areas and when it is desirable to achieve wide dispersal of low numbers of sterile flies, releases may be accomplished directly from aircraft or from ground vehicles calibrated to release the proper numbers of flies for the area involved. Higher losses would be expected when releasing from moving vehicles than from fixed stations because of the random placement of individuals in the environment, parts of which may be hostile. When pupae are being released, they may be placed in release boxes at sites that have been selected by ecologists taking into account the expected time of eclosion and taking proper measures against heat, rain, predators and parasites. The actual method of release and location of sites will have been predetermined by research teams in the field and chosen after consideration of local ecological factors involving the species under consideration. The distances between the release points will be determined considering the limits and possibilities of dispersal of the various species.

Tsetse flies are most likely to be infected by the brucei and congolense groups of trypanosomes if these are ingested with the first or second blood meal. It has been demonstrated that chemosterilized flies can transmit trypanosomes under laboratory conditions. For tsetse species important in transmitting these trypanosomes, delay of the release of sterile males until after the first blood meal has been taken would minimize the vector potential of the released flies. However, this advantage of adult releases would not apply to those tsetse flies that are only important for the transmission of T. vivax, as they can be infected at any age. Where reduction of the population with an insecticide is undertaken before release of sterile males, the total number of vectors need not exceed that of the population before the programme began. Where reduction is not undertaken, the total number of males will temporarily exceed the number normally present in the population; in this instance, it may be necessary to temporarily increase medical and veterinary vigilance due to the possibility of increased transmission. The risk of transmission of human trypanosomiasis may be reduced under these circumstances by releasing only adults that have had one or more blood meals prior to release. This has been done with G. palpalis (Clair et al 1976). In the case of G. morsitans however, it has

been shown that confinement of adults for short periods in the laboratory caused delayed flight muscle development (Dame and Ford, 1970; Langley, 1970; Bursell and Kuwenga, 1972).

E. Recommendations

1. For truly efficient and economical application of the SIT for a given species, information must be collected relating to suitable resting habitats and flight ranges under varying environmental conditions.
2. Consideration should be given to simplifying administrative procedures of transport, such as customs and border crossings, to avoid needless delays in delivery of fly shipments.
3. The creation of subsidiary centres for reception, stocking or distribution might facilitate the releases at the field trial site.
4. Since the methods of receipt and release will be determined to a large extent by local conditions, the release teams should appreciate the necessity of and strive to derive the best methods to permit the execution of their mission in the most rapid manner.
5. An effective aerial release method must be developed.
6. Determine the most advantageous stadium for release.
7. Evaluate optimum time between consecutive releases.
8. Evaluate optimum interval between release points in function of dispersal capability of flies.

VII. FIELD ASSESSMENT TECHNIQUES

Methods for obtaining accurate estimates of population density are not available for Glossina, although the apparent relative abundance of various species has been recorded on a seasonal basis in a large number of fly belts. The difficulty lies in the possible discrepancy between the actual number of flies present and the apparent number that is estimated from collection indices. These discrepancies result from diurnal and seasonal changes in fly behaviour. No mathematical conversion factor has been determined that will balance the seasonal changes in habitat and fly behaviour and provide an accurate estimate of population density for any species. Release-capture studies have been conducted to estimate population density and this technique may be adequate in most situations when fly density is reasonably high. However, even this technique falters if sampling methods are biased by fly behaviour, if the population is low and an inadequate number of marked flies are recaptured. Recent studies show that capture efficiency can be markedly increased, e.g. with use of electric nets (Vale, 1974); effective traps (Hargrove, 1977; Vale and Hargrove, 1979) and animal odours and certain chemicals (Vale, 1979). It may be possible to obtain extremely accurate population estimates, even when populations are low, by using a release-recapture technique. Thus, by conducting studies using this technique, in a variety of habitats and seasons, and concurrently monitoring the fly population by standard fly counts and examination of resting sites, seasonal conversion factors may be obtained for translating apparent density information directly into density estimates. Both apparent density data and population estimates derived from these data will be required to evaluate the effectiveness of a sterile release programme.

Regular surveillance should be initiated at least 12 months before releases start, with trypanosome infection rate dissections of flies before, during and after release operations. Surveillance should continue for a minimum period of 12 months after the last fly is captured. Periodic checks should be made for up to 5 years if required, depending upon the species.

Assessment of the effectiveness of a sterile release programme is complicated by the fact that tsetse flies can only be detected with great difficulty when the population is very low. As many of the following methods as possible should be evaluated before, during and after release programmes, and the best of them selected:

1. Detection by fly rounds, using man or animals as bait, or utilizing a vehicle with or without a specially constructed trapping device.
2. Traps in conjunction with olfactory attractants, if available.
3. Stationary point sampling methods, such as tethered bait animals.
4. Introduction of groups of various test animals to demonstrate disease transmission. This method is, however, of no value if more than one species of tsetse is present in the test area. It is recognized that disease transmission may be mechanical, diagnosis of some

trypanosome infections is cumbersome and some animals may not be sufficiently attractive for some fly species. This method will be of dubious value in riverine habitats. The need is also present for assessment of infection rate in the fly population.

5. Studies associated with prior reduction should include determination of fertility of populations in both the test and control areas. Suggestions for monitoring fertility during sterile release programmes include:
   a. the capture of wild females to assess their reproductive condition and/or potential;
   b. the capture of males for mating with virgin laboratory females to assess the reproductive potential in the field; and,
   c. the accurate recording at regular intervals of observed male:female ratios in wild populations .
6. The use of fly pickets on access roads and a continual marking programme outside the barrier zone could demonstrate the movement of flies into the release area and thus help to identify the source of fertile flies if they are found after completion of the release.

Inability to control human movement and activity severely restricts the feasibility of monitoring human sleeping sickness. Pupal surveys are not considered a suitable method of assessing the degree of control adhieved because of the difficulty of locating pupae even when fly density is high.

A. Recommendations

1. Renewed efforts should be made to develop and evaluate the potential of synthetic or natural attractants.
2. Further development is required in techniques of permanently marking flies either at the time of capture or at eclosion.
3. Pre-treatment assessment of populations should commence at least twelve months prior to the release of sterile males.
4. Post-treatment assessment should continue for at least twelve months following the cessation of sterile male releases.
5. A surveillance programme should be maintained to monitor the migration of flies into the treated zone.
6. Development of chemical attractants to attract large populations in the field would contribute to a more accurate assessment of field releases and their effect.
7. Establish techniques for determining when a population has been eradicated.

VIII. GENERAL RECOMMENDATIONS

1. There is ample scientific justification for aid-granting agencies to provide generous assistance in the form of funds, technical expertise and equipment to further research and development of the Sterile Insect Technique for the control of tsetse flies.

2. A number of laboratory colonies of tsetse flies have been successfully established in Europe and Africa. There is an urgent need for a larger number of such colonies, particularly in Africa, not only to permit research and development into the Sterile Insect Technique but also for work on a number of important problems such as epidemiology and transmission of trypanosomes. It is recommended that generous local, bilateral and multilateral assistance be given by national and international organizations towards the establishment of such colonies in Africa using the techniques already developed in Europe.

3. Because of the widespread occurrence and importance of Glossina species in Africa, it is urged that, whenever possible, programmes for tsetse fly mass rearing and application of the Sterile Insect Technique be organized and supported as regional programmes with the necessary training input to local personnel.

4. The Working Group wishes to encourage all tsetse field workers to collect, insofar as possible, the information (see sections II-VII) that would optimize the chances of success of a trial of the Sterile Insect Technique. This information could be circulated to all interested parties via the FAO/IAEA Information Circular on the Use of Radiation and Radioisotopes in Entomology, the WHO Trypanosomiasis Information Service, or the Tsetse and Trypanosomiasis Information and News Service.

5. Before launching a field trial of the sterility technique against tsetse flies, there must be a firm understanding between the research group and the local government concerning the exact nature, goal and duration of the programme.

6. With reference to research, the areas of highest priority which should be re-emphasized are:

a. Determination of what constitutes isolation; the definition of an effective barrier; determination of isolation parameters; the rate of re-infestation; and population movement.

b. Reduction of rearing through increased investigations, especially in the area of artificial diets, cage types and size, and the number of flies per cage.

c. Effects of sterilization must be elucidated. Further studies on field dispersal, competitiveness and vectorial capacity must be emphasized.

d. Development of autocidal techniques involving attractants/arrestants and chemosterilants to sterilize portions of wild populations, thus obviating the necessity for expensive rearing facilities.

7. Land use development plans should be drawn up for the area where the tsetse fly is to be controlled or eradicated. These plans should be designed so as to improve the natural resources of the area and to provide social and economic benefits.

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XI. LIST OF WORKING PAPERS PRESENTED AT ANTWERP

BAUER, B. — Performance of Glossina palpalis palpalis Rob.Desv. and G. morsitans morsitans Westw. fed on different blood sources.

CUISANCE, D., H. POLITZAR, M. CLAIR, Y. TAZE, and E. SELLIN — Release of sterile G. p. gambiensis at Bobo Dioulasso (Upper Volta): Effectiveness of sterile insect technique.

DAVEY, K.G. — Endocrine cycles in adults of Glossina austeni.

DOVER, G., and A. AMOS — A molecular approach to the elucidation of phylogenetic relationship in the genus Glossina.

GOODING, R.H. — Haemolysin and alkaline phosphate as digestive enzymes in Glossina morsitans morsitans Westw.

GOODING, R.H. and K.R. PARKER — Effects of feeding Glossina morsitans morsitans Westw. on previously exposed rabbits.

GOODING, R.H. and B.M. ROLSETH — Genetics and mating behaviour of Glossina morsitans morsitans Westw..

HAMANN, H-J. and K.H. IWANNEK — Sterilization of Glossina palpalis palpalis by Beta Irradiation.

ITARD, J. — Etat actuel des possibilités d'utilisation et des modalités d'application de la lutte contre les glossines par lâchers de males stériles.

ITARD, J. — Les élevages de glossines à Maisons-Alfort et à Bobo Dioulasso.

LANGLEY, P.A. — Application of sex recognition pheromone in Glossina to development of new strategies for control.

MEWS, A.R. — Problems and prospects for the in vitro feeding of tsetse.

NOGGE, G. — Elimination of symbionts of tsetse fly (G.m. morsitans) by feeding on rabbits immunized specifically with symbionts.

OFFORI, E.D. — The rearing of G. palpalis palpalis using (i) Rabbits, (ii) Guinea pigs.

OFFORI, E.D. — The use of alternate hosts for mass rearing of Glossina palpalis palpalis (Kaduna strain).

OFFORI, E.D. — Sterility and survival of gamma-irradiated Glossina palpalis palpalis (Kaduna strain).

TAKKEN, W. — Studies on the lipid reserves of Glossina palpalis palpalis which originated from two different scaring systems.

TENABE, S.O. and A.M.V. VAN DER VLOEDT — Effects of a sub-sterilizing dose of gamma radiation on the progeny of Glossina palpalis palpalis (Dipt.: Glossinidae) males.

TOBE, S.S. — Changes in free amino acids and peptides in the haemolymph of Glossina austeni during the reproductive cycle.

WETZEL, H. — The use of deep-frozen stored bovine blood for in vitro feeding of Glossina palpalis palpalis.

WETZEL, H. and M. TAHER — Use of equine, porcine and bovine blood for in vitro feeding of Glossina p. palpalis and G.m. morsitans.

WETZEL, H. and W. TAKKEN — In vitro feeding in the rearing of tsetse flies.

WILLIAMSON, D.L. — Production, sterilization and distribution of Glossina morsitans morsitans in the Tanzania field trial of the SIRM.

END